

## **AMENDMENTS TO THE CLAIMS**

### **LISTING OF CLAIMS:**

Claim 1. **(Withdrawn)** A cytochrome c oxidase complex having cytochrome c oxidase activity, which complex is obtainable by the isolation from a *Gluconobacter oxydans* DSM 4025 microorganism.

Claim 2. **(Withdrawn)** A cytochrome c oxidase complex according to claim 1, wherein the microorganism is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 3. **(Withdrawn)** A cytochrome c oxidase complex according to claim 1, wherein the complex has the following properties:

(a) comprising at least two core subunits of I (COI) and II (COII), wherein the apparent molecular mass of COI and COII are about 43  $\pm$  10 kDa and 36  $\pm$  10 kDa, respectively by SDS-PAGE; and

(b) providing an absorption spectrum showing an aa3-type cytochrome c oxidase peak at 605  $\pm$  1 nm in reduced minus oxidized difference spectrum.

Claim 4. **(Withdrawn)** A cytochrome c oxidase complex according to claim 1, wherein the isolated complex is substantially homologous to a native cytochrome

*c* complex from *Gluconobacter oxydans* DSM 4025 or a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 5. **(Withdrawn)** A cytochrome *c* oxidase complex according to any one of claims 1-4, which is a recombinant enzyme.

Claim 6. **(Withdrawn)** A cytochrome *c* oxidase complex according to claim 5 comprising a core subunit containing the amino acid sequence of SEQ ID NO: 2.

Claim 7. **(Withdrawn)** A cytochrome *c* oxidase complex according to claim 6 comprising an amino acid sequence having 85% or greater sequence identity with SEQ ID NO: 2, and having cytochrome *c* oxidase activity.

Claim 8. **(Withdrawn)** A cytochrome *c* oxidase complex according to claim 5 comprising at least one amino acid sequence selected from the group of SEQ ID NO: 4, 6 or 8.

Claim 9. **(Withdrawn)** A cytochrome *c* oxidase complex according to claim 8, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 4, 6 or 8, and is capable of providing the complex with cytochrome *c* oxidase activity.

Claim 10. **(Withdrawn)** A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

Claim 11. **(Withdrawn)** A recombinant polypeptide according to claim 10, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 2, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 12. **(Withdrawn)** A recombinant polypeptide according to claim 10, which is encoded by the polynucleotide sequence of SEQ ID NO: 1.

Claim 13. **(Withdrawn)** A recombinant polypeptide according to claim 12, wherein the polynucleotide sequence encodes SEQ ID NO: 2 or an amino acid sequence having at least 85% identity with SEQ ID NO: 2 and being capable of providing the complex with cytochrome c oxidase activity.

Claim 14. **(Withdrawn)** A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 4.

Claim 15. **(Withdrawn)** A recombinant polypeptide according to claim 14, wherein the polypeptide has an amino acid sequence that is at least 85%

identical to SEQ ID NO: 4, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 16. **(Withdrawn)** A recombinant polypeptide according to claim 14, which is encoded by a polynucleotide sequence of SEQ ID NO: 3.

Claim 17. **(Withdrawn)** A recombinant polypeptide according to claim 16, wherein the polynucleotide encodes SEQ ID NO: 4 or an amino acid sequence having at least 85% identity with SEQ ID NO: 4 and being capable of providing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 18. **(Withdrawn)** A recombinant polypeptide comprising an amino acid sequence of SEQ ID NOs: 6 or 8.

Claim 19. **(Withdrawn)** A recombinant polypeptide according to claim 18, wherein the amino acid sequence is at least 85% identical to either SEQ ID NOs: 6 or 8, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 20. **(Withdrawn)** A recombinant polypeptide according to claim 18, which is encoded by a polynucleotide selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 7.

Claim 21. **(Withdrawn)** A recombinant polypeptide according to claim 20 capable of providing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity, which is encoded by a polynucleotide selected from the group consisting of a polynucleotide encoding SEQ ID NO: 6, a polynucleotides encoding SEQ ID NO: 8, a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 6, and a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 8.

Claim 22. **(Currently amended)** A recombinant DNA ~~polynucleotide fragment~~ comprising the polynucleotide sequence of SEQ ID NO: 1.

Claim 23. **(Currently amended)** A recombinant DNA ~~polynucleotide fragment~~ comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2.

Claim 24. **(Currently amended)** A recombinant DNA ~~that encodes at least a part of core subunit I of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant~~ DNA comprising a polynucleotide sequence that encodes a polypeptide having an amino acid sequence that is at least 85% identical to SEQ ID NO: 2, wherein

the polypeptide forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit II.

Claim 25. (**Currently amended**) A recombinant DNA that ~~encodes at least a part of core subunit II of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising SEQ ID NO: 3, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit II that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit I.

Claim 26. (**Currently amended**) A recombinant DNA that ~~encodes at least a part of core subunit II of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit II that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit I.

Claim 27. (**Cancelled**).

Claim 28. (**Currently amended**) A recombinant DNA ~~that encodes at least a part of core subunit III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising SEQ ID NOs: 5 or 7, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit III that forms a complex having cytochrome c oxidase activity with *Gluconobacter oxydans* cytochrome c oxidase core subunits I and II.

Claim 29. (**Currently amended**) A recombinant DNA ~~that encodes at least a part of core subunit III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NOs: 6 or 8, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit III that forms a complex having cytochrome c oxidase activity with *Gluconobacter oxydans* cytochrome c oxidase core subunits I and II.

Claim 30. (**Cancelled**).

Claim 31. (**Currently amended**) An expression vector comprising a recombinant DNA according to any one of claims 22-26, 28 and 29 ~~that encodes at least a part of core subunits I, II and III of a cytochrome c oxidase complex~~

~~and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA comprising a polynucleotide sequence selected from the group consisting of polynucleotide sequences encoding the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, wherein the expression vector is suitable for expression in an organism.~~

Claim 32. **(Cancelled)**.

Claim 33. **(Original)** An expression vector according to claim 31, wherein the organism is a microorganism.

Claim 34. **(Original)** An expression vector according to claim 33, wherein the microorganism is a bacteria.

Claim 35. **(Original)** An expression vector according to claim 34, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 36. **(Currently amended)** An expression vector according to claim 35, wherein ~~wherein~~ the bacteria is *Gluconobacter oxydans* DSM 4025.



Claim 37. **(Previously presented)** An expression vector according to claim 34, wherein the bacteria is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 38. **(Original)** A recombinant microorganism comprising the expression vector of claim 31.

Claim 39. **(Original)** A recombinant microorganism comprising the expression vector of claim 36.

Claim 40. **(Currently amended)** A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 22-26, 28 and 29 that ~~encodes at least a part of core subunits I, II and III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA comprising a polynucleotide sequences selected from the group consisting of a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide sequence of SEQ ID NO: 3, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide sequence of SEQ ID NO: 5, a polynucleotide sequence that encodes the amino acid of SEQ ID NO: 6, a polynucleotide sequence of SEQ ID NO: 7, a polynucleotide~~

~~sequence that encodes the amino acid sequence of SEQ ID NO: 8, and combinations thereof.~~

Claim 41. **(Cancelled)**.

Claim 42. **(Original)** A recombinant microorganism according to claim 40, wherein the microorganism is a bacteria.

Claim 43. **(Currently amended)** A recombinant microorganism according to claim 40 42, wherein the microorganism is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 44. **(Currently amended)** A recombinant microorganism according to claim 40 43, wherein the microorganism is obtained from *Gluconobacter oxydans* DSM 4025.

Claim 45. **(Previously presented)** A recombinant microorganism according to claim 40, wherein the microorganism is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 46. (**Currently amended**) A process for producing a cytochrome c oxidase complex comprising:

(a) cultivating in a culture medium a recombinant microorganism ~~according to claim 40 comprising at least one recombinant DNA that encodes at least a part of core subunits I, II and III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA comprising polynucleotide sequences selected from the group consisting of SEQ ID NO: 1, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 5, a polynucleotide sequence that encodes the amino acid of SEQ ID NO: 6, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, SEQ ID NO: 7, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 8, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8, and combinations thereof; and~~

(b) recovering cytochrome c oxidase from the culture.

Claim 47. **(Original)** A process according to claim 46, wherein the recombinant microorganism is a bacteria.

Claim 48. **(Currently amended)** A process according to claim ~~46~~ 47, wherein the microorganism ~~bacteria~~ is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 49. **(Currently amended)** A process according to claim ~~46~~ 48, wherein the microorganism is obtained from *Gluconobacter oxydans* DSM 4025.

Claim 50. **(Currently amended)** A process according to claim ~~46~~ 49, wherein the microorganism is a biologically and/or taxonomically homogeneous culture ~~biological or taxonomic homolog~~ of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 51. **(Withdrawn)** A process for producing 2-keto-L-gluconic acid (2-KGA) from L-sorbose or D-sorbitol comprising:

(a) cultivating in a culture medium a recombinant microorganism comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting a polynucleotide sequence of SEQ ID NO: 1, a

polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment of SEQ ID NO: 3, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, the polynucleotide fragment of SEQ ID NO: 5, a polynucleotide fragment that encodes the amino acid of SEQ ID NO: 6, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, a polynucleotide fragment of SEQ ID NO: 7, and a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 8, and a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8 and capable of expressing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity; and

(b) recovering 2-KGA from the culture medium.

Claim 52. **(Withdrawn)** A process according to claim 51, wherein the recombinant microorganism is a bacteria.

Claim 53. **(Withdrawn)** A process according to claim 52, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*,

*Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 54. **(Withdrawn)** A process according to claim 53, wherein the microorganism is *Gluconobacter oxydans* DSM 4025.

Claim 55. **(Withdrawn)** A process according to claim 54, wherein the microorganism is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 56. **(Withdrawn)** A cytochrome c oxidase complex comprising a core subunit containing a polypeptide sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8, fragments of SEQ ID NO:2 capable of providing the said complex with cytochrome c oxidase activity, and a polynucleotide sequence that encodes a polypeptide that is capable of providing the complex with cytochrome c oxidase activity, and which polynucleotide hybridizes under high stringency hybridization and wash conditions to a polynucleotide sequence encoding SEQ ID NO:2, 4, 6 or 8.

Claim 57. **(Currently amended)** A recombinant DNA ~~that encodes at least a part of core subunit I of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant~~

DNA comprising a polynucleotide sequence that hybridizes to the complementary strand of SEQ ID NO: 1 under high stringency conditions comprising ~~[[()]]~~ overnight incubation in 6X SSC, 0.5% SDS, 100 ug/ml denatured salmon sperm DNA, 50% formamide, ~~with gentle rocking~~ at 42°C; followed by a first wash in 2X SSC, 0.5% SDS at room temperature for 15 minutes; followed by a second wash in 0.1X SSC, 0.5% SDS at room temperature for 15 minutes ~~[[()]]~~, wherein the recombinant DNA encodes a polypeptide that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit II.

Claims 58 and 59. **(Cancelled).**

Claim 60. **(Currently amended)** A recombinant DNA according to claim 24, wherein the complex comprises ~~is isolated from a *Gluconobacter oxydans* DSM 4025 cytochrome c oxidase core subunits I and II microorganism.~~

Claim 61. **(Currently amended)** A recombinant DNA according to claim 24, wherein the complex comprises ~~has the following properties:~~

(a) at least a core subunit I (COI) and a core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE~~[[()]]~~ and

(b) the complex displays an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.

Claim 62. (**Previously presented**) A recombinant DNA according to claim 24, wherein the complex is a recombinant enzyme.

Claim 63. (**Currently amended**) A recombinant DNA according to claim 24, wherein core subunit II ~~the complex comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO: 4, 6, or 8, and amino acid sequences that are 85% identical to SEQ ID NO: 4, 6 or 8.~~

Claim 64. (**Currently amended**) A recombinant DNA according to claim 26 27, wherein the subunits are ~~complex is isolated from a *Gluconobacter oxydans* DSM 4025~~ cytochrome c oxidase subunits ~~microorganism.~~

Claim 65. (**Currently amended**) A recombinant DNA according to claim 26 27, wherein the complex comprises ~~has the following properties:~~

(a) at least a core subunit I (COI) and a core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE~~[[;]]~~ and



(b) the complex displays an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.

Claim 66. **(Currently amended)** A recombinant DNA according to claim 26 27, wherein the complex is a recombinant enzyme.

Claim 67. **(Currently amended)** A recombinant DNA according to claim 26 27, wherein core subunit I ~~the complex~~ comprises ~~at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2 and amino acid sequences that are 85% identical to SEQ ID NO: 2.~~

Claim 68. **(Currently amended)** A recombinant DNA according to claim 29 30, wherein the subunits are ~~complex is isolated from a~~ *Gluconobacter oxydans* DSM 4025 cytochrome c oxidase subunits ~~microorganism~~.

Claim 69. **(Currently amended)** A recombinant DNA according to claim 29 30, wherein ~~the complex has the following properties:~~

(a) ~~at least core subunit I (COI) and core subunit II (COII),~~  
wherein the apparent molecular masses of core subunits I and II ~~COI and COII~~ are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE[[:]] and

(b) the complex displays ~~providing~~ an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.

Claim 70. **(Currently amended)** A recombinant DNA according to claim 29 ~~30~~, wherein the complex is a recombinant enzyme.

Claim 71. **(Currently amended)** A recombinant DNA according to claim 29 ~~30~~, wherein core subunit I ~~the complex~~ comprises ~~at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2 and amino acid sequences that are 85% identical to SEQ ID NO: 2.~~

Claim 72. **(Currently amended)** An expression vector comprising at least one recombinant DNA according to any one of claims 60, 64 and 68 ~~claim 32~~, wherein ~~the complex is isolated from a *Gluconobacter oxydans* DSM 4025 microorganism.~~

Claim 73. **(Currently amended)** An expression vector comprising at least one recombinant DNA according to any one of claims 61, 65 and 69 ~~claim 32~~, wherein ~~the complex has the following properties:~~

~~(a) — at least core subunit I (COI) and core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE; and~~

~~(b) — an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.~~

Claim 74. **(Currently amended)** An expression vector comprising at least one recombinant DNA according to any one of claims 62, 66 and 70 ~~claim 32,~~ wherein ~~the complex is a recombinant enzyme.~~

Claim 75. **(Currently amended)** A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 60, 64 and 68 ~~claim 41,~~ wherein ~~the complex is isolated from a *Gluconobacter oxydans* DSM 4025 microorganism.~~

Claim 76. **(Currently amended)** A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 61, 65 and 69 ~~claim 41,~~ wherein ~~the complex has the following properties:~~

~~(a) — at least core subunit I (COI) and core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE; and~~

(b) ~~an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.~~

Claim 77. **(Currently amended)** A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 62, 66 and 70 claim 41, wherein the complex is a recombinant enzyme.

Claim 78. **(Currently amended)** A process for producing a cytochrome c oxidase complex comprising: according to claim 46, wherein the complex is isolated from a *Gluconobacter oxydans* DSM 4025 microorganism

(a) cultivating in a culture medium a recombinant microorganism according to claim 75; and

(b) recovering cytochrome c oxidase from the culture.

Claim 79. **(Currently amended)** A process for producing a cytochrome c oxidase complex comprising: according to claim 46, wherein the complex has the following properties:

(a) cultivating in a culture medium a recombinant microorganism according to claim 76 at least core subunit I (COI) and core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE; and

(b) recovering cytochrome c oxidase from the culture an  
~~absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$~~   
~~nm in a reduced minus oxidized difference spectrum.~~

Claim 80. **(Currently amended)** A process for producing a cytochrome c  
oxidase complex comprising: ~~according to claim 46, wherein the complex is a~~  
~~recombinant enzyme~~

(a) cultivating in a culture medium a recombinant microorganism  
according to claim 77; and

(b) recovering cytochrome c oxidase from the culture.